PHYTOCHEMICAL ANALYSIS AND ANTIMICROBIAL ACTIVITY OF LEAF EXTRACT OF RUTA GRAVEOLENS L.,

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ABSTRACT

Medicinal plants have been used as traditional treatments for numerous human diseases for thousands of years. Ruta graveolens is commonly known as Rue. It belongs to the Rutaceae family. The present study was focused on preliminary phytochemical analysis of different extracts (Ethanol, methanol, chloroform, petroleum ether, hexane and acetone) of Ruta graveolens leaves. The result showed the presence of alkaloids, flavonoids, betacyanin, fixed oils, resins, saponin. Antibacterial activity of ethanol and methanol extract of R. graveolens leaves against the microbes Escherichia coli, Staphulococcus aureus. Showed maximum zone of inhibition against S.aureus (23mm) when 100µl of ethanol extract was used. Possibilities of peptides in the leaf protein of Ruta graveolens as antimicrobial principles is suggested.

KEYWORDS: Ruta Graveolens, Phytochemical Screening, Antibacterial Activity

INTRODUCTION

Medicinal plants were used by people of ancient culture without knowledge of their active ingredients. The use of medicinal herb in the treatment and prevention of diseases is attracting attention by scientists World Wide. This is also supported by World Health Organization in its quest to bring primary health care to the people.

Rutaceae is one of the largest family, consisting of more than 150 genera and 1600 difference species of shrubs and small trees that grow mostly in temperate countries of the old and new world. The most common medicinal plant of this family is Ruta graveolens L., which is commonly known as ‘Aruvada’ in Tamil and ‘Rue or Sudab’ in hindi.

It is a perennial herbaceous evergreen shrub of one meter tall, and has considerable medicinal importance with a characteristic grayish color and a sharp unpleasant odour. The leaves are small, oblong, deeply divided, pinnate, glandular dotted. It is native to Europe, specially the Mediterranean region, widely distributed in all the temperates and largely in tropical and subtropical regions of the world.
The present study was designed to investigate various phytochemical analysis and antimicrobial activity in different extracts of Ruta graveolens leaves.

Materials and Methods:

Collection of plant:
The plant *Ruta graveolens* belonging to the family Rutaceae, was collected from Ooty, TamilNadu. Leaf of *R. graveolens* were collected and kept for further study.

Preparation of plant extract:
5g of powdered leaf material was each separately dispersed in 50ml of each ethanol, methanol, chloroform, petroleum ether, hexane and acetone. The solution was left to stand at room temperature for 24hrs and was filtered with Whatman No. 1 filter paper. The filtrate was used for the phytochemical screening using the following tests.

Preliminary phytochemical analysis of different crude extracts
Extracts were tested for the presence of active principles such as Alkaloids, Glycosides, Carbohydrates, Flavonoids, Quinones, Betacyanin, Coumarin, Protein, Resins, Phenols Saponins, Fixed oils and fats, Anthraquinones glycosides, Naphthaquinones and Phlobatannins.

Preliminary Phytochemical screening

Test for Alkaloids (Kokate *et al.*, 2005)
- **Mayer’s Reagent**: 1ml of extract was added to 2ml of Mayer’s reagent and development of cream precipitate indicates the presence of alkaloids.
- **Wagner’s Reagent**: 1ml of extract was added to 2ml of Wagner’s reagent and development of reddish brown precipitate indicates the presence of alkaloids.
- **Tannic acid test**: Alkaloids give buff colour precipitate with tannic acid test.

Test for Flavonoids (Vasandha *et al.*, 2011)
- **Alkaline Reagent**: Extracts were treated with few drops of Sodium hydroxide solution. Formation of intense yellow colour, which becomes colourless on addition of dilute acid, indicates the presence of flavonoids.
- **Lead acetate solution test**: Test solution when treated with few drops of lead acetate solution would result in the formation of yellow precipitate.
- **Zinc hydrochloric acid test**: To the test solution, mixture of zinc dust and conc. HCl acid was added. It gives red colour after few minutes.
**Flavanols:** To the extract a pinch of Boric acid and few drops of Acetic acid when added, bright yellow colour formation with green fluorescence indicate flavonols.

**Rao & Sheshadri test:** To the extract, few drops of con. Nitric acid was added. Brilliant blue colour indicates the presence of phloroglucinol derived flavanones.

**Test for glycosides (Shilika and Vijayalaxmi, 2012)**

**Sulphuric acid test:** To 1ml extract few drops of conc. sulphuric acid was added and mixed well. The contents were allowed to stand for few minutes. Appearance of reddish brown precipitate indicates the presence of glycosides.

**Test for Carbohydrates (Kokate et al., 2005)**

**Fehling’s test:** Test solution was mixed with few drops of Fehling’s reagent and boiled in water bath, observed for the formation of blue colour.

**Test for Quinones (Anjali and Sheetal, 2013)**

Dilute NaOH was added to 1ml of crude extract. Blue green or red coloration indicate the presence of quinones.

**Test for Betacyanin (Sofoware, 1982)**

To 2ml of plant extract, 1ml of 2N Sodium hydroxide was added and heated for 5min at 100°C. Formation of yellow colour indicates the presence of betacyanin.

**Test for Coumarin (Sofowara, 1982)**

To 1ml of extract, 1ml of 10% Sodium hydroxide was added. Formation of yellow colour indicates the presence of coumarin.

**Test for Protein (Vasandha et al., 2011)**

**Biuret test:** The extract was treated with 1ml of 10% Sodium hydroxide solution and heated. To this a drop of 0.7% copper sulphate was added. Formation of purplish violet colour indicate the presence of proteins.

**Xanthoprotein:** 3ml of test solution was taken in a test tube. To this 1ml of conc. Sulphuric acid was added along the sides of the test tube. Yellow precipitate has to be observed.

**Trichloroacetic acid:** To the solution if trichloroacetic acid is added, precipitate is formed.

**Test for Resins (Vasandha et al., 2011)**

**Acetone- water test:** Extracts were treated with acetone and small amount of water was added and shaken. Appearance of turbidity indicates the presence of resins.
Test for phenols (Vasandha et al., 2011)

Ferric chloride test: Extracts were treated with few drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenols.

Test for Saponins (Kokate et al., 2005)

Foam test: To 2ml of extract was added 6ml of water in a test tube. The mixture was shaken vigorously and observed for the formation of persistent foam that confirms the presence of saponins.

Test for fixed oils and fats (Vasandha et al., 2011)

Stain test: Small quantities of extracts were pressed between two filter papers. An oily stain on filter paper indicates the presence of fixed oils.

Anthraquinones glycosides (Kokate et al., 2005)

Hydroxy-anthraquinones: The sample when treated with potassium hydroxide solution red colour is produced.

Naphthaquinones (Kokate et al., 2005)

Junglone test: Sample when treated with 2ml of chloroform extract and 2ml of ethyl ether with dilute ammonia solution. Pink colour formation indicates naphthoquinones.

Phlobatannins (Anjali, 2013)
The crude extract of the plant sample was boiled with 2% aqueous HCl. The deposition of a red precipitate was taken as evidence for the presence of phlobatannins.

Antimicrobial activity

Crude protein preparation for Antibiotic assay

Acetone method: (Francis et al., 2014)

5g of powdered leaf plant material were each separately dispersed in 50ml of each ethanol, methanol. The solution was left to stand at room temperature for 24hrs and was filtered with whatman No. 1 filter paper. The filtrate was dried and the residue was mixed with ethanol and methanol separately. Equal volumes of acetone and the filtrate extract were mixed and vortexed. This mixture was kept overnight at 4°C and then centrifuged at 15000 rpm for 15 minutes. The supernatant was allowed to dry until the formation of a pellet. Complete desiccation of the protein pellet was avoided.

Antibacterial activity (Well diffusion method)
The antibacterial assays were done on bacterial organisms like multiple drug resistant hospital isolates of Escherichia coli (E.coli), and Staphylococcus aureus by well diffusion method.
Nutrient Agar medium was prepared and autoclaved. After solidification, 24 hour old culture of each of the test organisms grown in Agar medium was swabbed. Wells of diameter 10mm diameter were punched into each swabbed plates. Different concentrations Viz., 20, 40, 60, 80 and 100µl of crude protein extracts of leaf of *R. graveolens* were poured in the wells. Ethanol, methanol and 20 µl of Antibiotic drug streptomycin were used as controls. Bacterial plates were incubated at 37°C room temperature for 24 hours. The plates were observed for inhibition zones and the diameters of the inhibition zone was measured.

### EXPERIMENTAL RESULTS

**Table-1 Qualitative phytochemical analysis of Leaf extract of *Ruta graveolens***

<table>
<thead>
<tr>
<th>S.No</th>
<th>PhytoConstituents</th>
<th>Ethanol</th>
<th>Methanol</th>
<th>Chloroform</th>
<th>Petroleum Ether</th>
<th>Hexane</th>
<th>Acetone</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>1.</strong></td>
<td>Alkaloids</td>
<td>Mayer’s test</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Wagner’s test</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Tannic acid test</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td><strong>2.</strong></td>
<td>Flavonoids</td>
<td>Flavonols</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Tannic acid</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Zinc hydrochloride</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>3.</strong></td>
<td>Carbohydrates And Glycosides</td>
<td>Fehling’s test</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Sulphuric acid test</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>4.</strong></td>
<td>Protein</td>
<td>Xanthoproteic test</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Trichloro acetic acid test</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Biruet test</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>5.</strong></td>
<td>Quinones Test</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>6.</strong></td>
<td>Coumarin Test</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><strong>7.</strong></td>
<td>Phlobatannins</td>
<td>Precipitate test</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>8.</strong></td>
<td>Betacyanin</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
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<tr>
<td><strong>9.</strong></td>
<td>Fixed Oil And Fat</td>
<td>Stain test</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
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<tr>
<td><strong>10.</strong></td>
<td>Resins</td>
<td>Acetone water test</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td><strong>11.</strong></td>
<td>Phenol</td>
<td>Ferrie chloride test</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>12.</strong></td>
<td>Saponin</td>
<td>Foam test</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><strong>13.</strong></td>
<td>Anthra Quinone</td>
<td>Hydroxy anthra quinines</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>14.</strong></td>
<td>Naptha Quinone</td>
<td>Jungle test</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

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### Table 2: Antibacterial activity of crude protein of ethanol extract of leaf of Ruta graveolens

<table>
<thead>
<tr>
<th>S.No</th>
<th>Name of the organisms</th>
<th>Control -20µl Antibiotic</th>
<th>Zone of inhibition (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>20µl</td>
</tr>
<tr>
<td>1.</td>
<td><em>Escherichia coli</em></td>
<td>35mm</td>
<td>-</td>
</tr>
<tr>
<td>2.</td>
<td><em>Staphylococcus aureus</em></td>
<td>30mm</td>
<td>15mm</td>
</tr>
</tbody>
</table>

### Table 3: Antibacterial activity of crude protein of methanol extract of leaf of Ruta graveolens

<table>
<thead>
<tr>
<th>S.No</th>
<th>Name of the organisms</th>
<th>Control-20µl Antibiotic</th>
<th>Zone of inhibition (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>20µl</td>
</tr>
<tr>
<td>1.</td>
<td><em>Escherichia coli</em></td>
<td>35mm</td>
<td>-</td>
</tr>
<tr>
<td>2.</td>
<td><em>Staphylococcus aureus</em></td>
<td>30mm</td>
<td>-</td>
</tr>
</tbody>
</table>

**PLATE 1**

Antibacterial activity of crude protein from ethanol and methanolic extract leaf of *R. graveolens*

**Control (Escherichia coli)**

**Control (Staphylococcus aureus)**
Results and Discussion:

Qualitative phytochemical analysis

Phytochemical analysis conducted on the plant extracts revealed the presence of constituents which are known to exhibit medicinal as well as physiological activities. The phytochemical characteristics of the leaf extract of Ruta graveolens investigated are summarized in table-1.

The leaves extracts of R. graveolens was collected, dried and extracted with different solvents such as ethanol, methanol, chloroform, petroleum ether, hexane and acetone. The results showed the presence of medicinally active constituents like flavonoids and resins in all the extracts tested. Ethanol, methanol and petroleum ether extracts showed the presence of phlobatannins, fats and fixed oils, resins, flavanols and betacyanin. Alkaloids are present only in the methanol, chloroform, petroleum ether and hexane extracts. Presence of carbohydrates were found in ethanol and methanol extracts. Anti-inflammatory effect of Ruta graveolens has been reported by Raghav et al., (2006) Similarly the antioxidant activity of this plant has been attributed to its flavonoids and phenolic contents by Proestos et al., (2006)
Diameter of growth inhibition zone by crude protein of *Ruta graveolens* leaf extracts on bacteria

The present study was conducted to evaluate the protein based antibacterial activity of leaf extract of *Ruta graveolens*. Different concentration viz 20, 40, 60, 80, 100µl of the crude protein extract of leaf was analyzed for its antibacterial activity against *Escherichia coli* and *Staphylococcus aureus* by well diffusion method. The zone of inhibition was higher in ethanol extracts (23mm) when 100µl of the extracts was used against *S. aureus*. In *E. coli* the minimum inhibition started at the concentration of 60µl, and not much difference could be seen in higher concentration. i.e. 100µl (Table 2,3 and Plate 1). The potent antimicrobial activity of *Ruta graveolens* leaves has been reported by Olive *et al.*, (2003). They have attributed the antifungal activity of ethyl acetate extract of *Ruta graveolens* leaves to furanocoumarins. The present study indicates the possibilities of peptides in the leaf extract of *Ruta graveolens* as active principles which should be further explored.

CONCLUSION

*Ruta graveolens* plant belongs to Rutaceae family. These plants have a good effects like cytotoxic, hypotensive properties. The preliminary phytochemical analysis for alkaloids, flavonoids, carbohydrates, proteins, quinonens, coumarin, betacyanin, fixed oils, resins, phenol, saponin, anthra quinone, were done with leaf powder of *R. graveolens*. The results of present study concluded that the different extracts of *R. graveolens* shows in vitro antibacterial activity against human pathogens like *E.coli, S.aureus*. Among the two different extracts of *Ruta graveolens* L the methanol extracts shows good antibacterial activity against test pathogens.

**References**


